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(54) Title: NOVEL REAGENT FOR TUMOUR IMAGING AND THERAPY

(57) Abstract

Novel oligopeptides are disclosed for *in vivo* tumour imaging and therapy. The novel oligopeptides are oligopeptides containing from 4 to 50 peptide units containing as a characteristic triplet therein the amino acid sequence leu-asp-val (LDV), which triplet provides the oligopeptide with a strong *in vivo* binding affinity for LDV binding sites on tumours and other tissues. For diagnostic purposes the oligopeptides are labelled with a radioactive label, e.g. ^{125}I, whilst for therapeutic purposes the oligopeptides are chemically linked to or conjugated with a cytotoxin, such as the ricin A-chain.

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NOVEL REAGENT FOR TUMOUR IMAGING AND THERAPY

FIELD OF INVENTION

This invention relates to in vivo tumour imaging and therapy, and more particularly to a novel reagent for use therein, diagnostic and therapeutic compositions containing that reagent, and methods of tumour imaging and therapy utilising the reagent.

BACKGROUND INFORMATION AND PRIOR ART

In the cause of improving the diagnosis and treatment of cancer numerous attempts have been made to target imaging agents, e.g. radioactive isotopes, and therapeutic reagents onto tumours in vivo using anti-tumour monoclonal antibodies, Mabs, see for example published International Applications WO 89/00583 and WO 90/09197. Because of the size of the average Mab, diffusion rates of, for example, a radio-labelled antibody to the site of a tumour are very low, and the same applies to conjugates formed from a cytotoxic reagent and an anti-tumour antibody.

Tumour imaging and therapy using Mabs is therefore inherently a slow process, often taking several hours, whereas ideally one would like to have the process complete in minutes, rather than hours, particularly in the case of tumour imaging and diagnosis.

In J. Immunol., 145, No. 1, 59-67, 1990, Shimizu et al have reported on the co-stimulation of proliferative responses of resting $CD4U^{+}$T-cells by the interaction of the extracellular matrix (ECM) proteins fibronectin (FN) and laminin (LN) with the VLA integrins VLA-4 and VLA-5 (in the case of fibronectin) or VLA-6 (in the case of laminin) expressed by resting human T-lymphocytes, and have shown inter alia that the 12-amino acid peptide

L H G P E I L D V P S T

(leu-his-gly-pro-glu-ile-leu-asp-val-pro-ser-thr)

is an effective T-cell adhesion inhibitor and an effective blocking agent for OKT3/FN T-cell proliferation, but does not go beyond a mere investigation into the role of cell adhesion molecules (CAMs) in T-cell recognition and activation. Thus, Shimizu et al suggest no practical

outcome or industrial utility resulting from their investigations.

In J. Biol. Chem., 266, No. 6, 3579-3585, 1991, Mould et al have reported their studies of the inhibition of the interaction of the integrin heterodimer $\alpha_4\beta_1$ with the CS1 and CS5 sites in the IIICS region of fibronectin, which interaction is believed to play an important role in melanoma cell adhesion, and have shown that the tripeptide X-asp-Y, where X is glycine, leucine or glutamic acid, and Y is serine or valine, represents a minimum recognition sequence within the CS1 site of the fibronectin for the integrin $\alpha_4\beta_1$. Inter alia, those studies utilised synthetic CS1 and KKT-CS1-VQK peptides, viz:

CS1

D E L P Q L V T L P H P N L H G P
asp-glu-leu-pro-gln-leu-val-thr-leu-pro-his-pro-asn-leu-his-gly-pro-
E I L D V P S T
glu-ile-leu-asp-val-pro-ser-thr

KKT-CS1-VQK

K K T D E L P Q L V T L P H P N L
lys-lys-thr-asp-glu-leu-pro-gln-leu-val-thr-leu-pro-his-pro-asn-leu-
H G P E I L D V P S T V Q K
his-gly-pro-glu-ile-leu-asp-val-pro-ser-thr-val-gln-lys

but again no consequential commercial utility is suggested for such LDV (leu-asp-val) containing peptides.

Subsequent studies of similar kind confirming the significance of the LDV triplet as the minimum recognition site for the $\alpha_4\beta_1$ integrin, as opposed to the RGDS sequence which constitutes the minimum recognition site for the $\alpha_5\beta_1$ integrin, those two minimum recognition sites occuring in different domains of the fibronectin (FN) molecule, viz. the alternatively spliced type III connecting segment (IIICS) and the central cell binding domain respectively, are reported by Komoriya et al in J. Biol. Chem., 266, No. 23, 15075-15079, 1991, but published after the present priority date.

Earlier studies into the cell binding activity of fibronectin are reported by Pierschbacher and Ruoslahti (Nature, 309, 30-33, 1984) and by Kloczewiak et al in Biochemistry, 28, 2915-2919, 1989.

Pierschbacher et al recognise the importance of the RGDS sequence as a recognition site in the cell adhesion activity of fibronectin, but report that they were unable to obtain any inhibition of cell adhesion using either soluble fibronectin or by peptides not containing the RGDS sequence. Similarly Kloczewiak et al, investigating the essential role of the terminal region of the fibrinogen γ-chain in the interaction of human fibrinogen with activated platelets, showed that the synthetic dodecapaptide:

H H L Q L L K Q L L D V

his-his-leu-gln-leu-leu-lys-gln-leu-leu-asp-val

being an analogue of the γ400-411 FN residue, and containing the LDV triplet, achieved 50% inhibition of fibrinogen binding to activated platelets, but only at concentrations greater than 500μM.

In contrast to both Shimizu et al and Mould et al, EP-A-0428266 identifies as potential anti-cancer agents, peptides based on the functional domains of fibronectin, more particularly the cell binding domains and the heparin binding domains, amino acid residues 1239-1515 and 1690-1960 respectively (EP-A-0428266, SEQ ID Nos. 2 and 3). According to EP-A-0428266, these fibronectin fragments show anti-cancer activity in mice by inhibiting metastasis, and may be used singly or in the form of a chimeric peptide comprising both sequences linked by a linker, e.g. a methionine residue, EP-A-0428266 SEQ ID No. 4. In either event, the peptide fragment is of substantial size: 277 amino acid residue in the case of the cell binding domain FN fragment, and 271 in the case of the heparin binding FN fragment, making a total of 549 amino acid residues in the chimeric peptide. Such fragments are therefore still of substantial size compared to the oligopeptides used in the present invention, and can be expected still to show many of the disadvantages of the Mab based anti-cancer agents, namely low diffusion and clearance rates. Not only that, but none of the FN fragments disclosed as anti-cancer agents in EP-A-0428266 contain the amino acid triplet which is characteristic of the oligopeptides used in accordance with the present invention, that is to say the leu-asp-val (LDV) triplet.

More recently, in published International Patent Application WO

90/15858, the present inventor has disclosed novel peptides comprising an RGD sequence (i.e. the sequence: arg-gly-asp) primarily for in vivo thrombus imaging, but also potentially useful for in vivo tumour imaging and therapy, and capable of binding to tumours in vivo via RGD binding sites on the surface of the tumour. In the case of thrombus imaging the peptide binds to RGD recognition and binding sites in the GPIIb/IIIa (glycoprotein-fibrinogen receptor) complex present on the membrane surface of activated platelets, and which contain the fibrinogen binding domains and which are involved in the aggregation of the activated platelets to form the thrombus.

OBJECTS OF THE PRESENT INVENTION

The present invention seeks to overcome the above-mentioned disadvantages of monoclonal antibody (Mab) based cytotoxic and diagnostic reagents by providing novel oligopeptide based reagents having specific binding properties to tumour associated binding sites, and which are rapidly transported to the tumour site following injection, with equally rapid clearance of unbound reagent from the body following administration, thus considerably reducing treatment times and reducing the effective dosage of potentially highly toxic reagents, whether administered for diagnostic or therapeutic purposes.

Further objects of the present invention are to provide tumour diagnostic and therapeutic compositions containing such reagents, and methods of diagnostically imaging or treatment of tumours using such reagents.

SUMMARY OF PRESENT INVENTION

In accordance with the present invention the above objectives are achieved using a synthetic oligopeptide of from 4 to about 50 peptide units and containing the sequence

L D V
leu-asp-val

referred to herein as the LDV sequence, which sequence binds the oligopeptide in vivo to LDV binding sites on the tumour.

Because of their relatively small size, such oligopeptides

diffuse rapidly throughout the body and, equally importantly, are rapidly cleared from the body by ordinary metabolic and/or excretory processes, leaving behind only bound oligopeptide bound to the tumour or to other tissue structures containing an LDV binding site. This rapid clearance of unbound oligopeptide from the body is particularly important in the case of tumour imaging using a radio-labelled oligopeptide, since it permits visualisation of local concentrations of bound radio-labelled oligopeptide by conventional radiological techniques within a relatively short period of time following intravenous administration of the diagnostic reagent, i.e. the radio-labelled oligopeptide.

The rapid clearance of unbound oligopeptide from the body is also invaluable in reducing possible toxic side effects of cytotoxic drugs used in tumour therapy regimes and administered, in accordance with the concepts of this invention, as a conjugate formed from the cytotoxic reagent and an oligopeptide of from 4 to about 50 peptide units and containing the sequence

L D V

leu-asp-val

BRIEF DESCRIPTION OF DRAWINGS

The binding capacity of radio-labelled peptides according to the invention to tumour cells, both in vitro and in vivo is illustrated by the accompanying drawings, in which:

Figure 1 is a bar chart illustrating the binding capacity of the radio-labelled peptide ^{125}I-YLDVY to tumour cells from cell lines T47D, LoVo, HT29, HT1080, HEp-2, EJ28 and A431, in vitro;

Figure 2 presents similar data in respect of the radio-labelled peptide ^{125}I-YGGLDVGLDVGGY;

Figure 3 presents similar data in respect of the radio-labelled peptide ^{125}I-LDVGGGGSY; and

Figure 4 is a bar chart illustrating the in vivo retention of the radio-labelled peptide ^{125}I-YGGLDVGLDVGGY by nude mice bearing HEp-2 and HT29 tumours.

DETAILED DESCRIPTION

In accordance with this invention, therefore, there are provided diagnostic and therapeutic reagents, primarily for in vivo tumour imaging and therapy, but also useful in targeting other pathological tissues containing an LDV-binding site, such reagents comprising an oligopeptide of from 4 to about 50 peptide units and containing as a triplet therein the sequence

L D V
leu-asp-val

and to which oligopeptide there is attached either a radioactive label or cytotoxin.

As to the actual size of the oligopeptide used in accordance with the present invention, this will be determined largely by economic considerations: generally in the construction of oligopeptides on a peptide synthesizer cost is directly proportional to length. For synthetic oligopeptides therefore, 50 peptide units represents the maximum chain length that is likely to be viable economically, although in practice very much shorter chain lengths are the more likely, e.g. from 4 to 30, down to 4 to 20 or 4 to 15, or as little as 4 to 10. Particularly preferred sequences are the CS1 sequence of fibronectin, i.e. a 25-unit oligopeptide having the sequence SEQ ID No. 1, and sub-units thereof containing the LDV triplet. Other suitable oligopeptides include the five unit peptide:

Y L D V Y
tyr-leu-asp-val-tyr

the nine unit peptide:

L D V G G G G S Y
leu-asp-val-gly-gly-gly-gly-ser-tyr

and the thirteen unit peptide: SEQ ID No. 2.

The CS1 sequence of fibronectin, SEQ ID No. 1, is known to bind to the cell adhesion molecule (CAM) VLA-4 expressed in vitro by many tumour cells and by resting $CD4^{+}$T-cells (see Shimizu et al above). It

is now established that the CS1 sequence of fibronectin and other LDV-containing peptides bind strongly to tumour cells in vivo, thus providing a method of targeting imaging or therapeutic reagents onto those cells.

A variety of different techniques are available for attaching a radioactive label to the oligopeptide, and a variety of different labels are available for this purpose. Amongst those there may be mentioned, in particular: technetium-99m, iodine-123 or iodine-125 and indium-111. In order to attach the label (or for that matter, a cytotoxic reagent) the oligopeptide will usually be provided with a reactive terminal amino acid residue such as a terminal tyrosine, histidine, lysine or cysteine residue. When using ^{99m}Tc as the label this may be attached via a cysteine residue, ^{125}I via a tyrosine residue, and ^{111}In via a lysine residue. Attachment of ^{125}I to an oligopeptide via a tyrosine residue is described in WO 90/15818, and the same procedure may be used herein. Other suitable techniques are described in Science 220, 613-615; Int. J. Nucl. Med. Biol., 12, 3-8; J. Nucl. Med., 26, 293-299 and J. Nucl, Med., 27, 685-693, incorporated herein by reference.

In the alternative, there may be attached to the oligopeptide, in place of the radioactive label, a cytotoxic agent, such as ricin or a derivative or component thereof, particularly the ricin A-chain. Methods of attaching a cytotoxic agent, such as ricin A-chain, to the oligopeptide will depend on the particular cytotoxin to be used, and the functional groups contained therein and by means of which the cytotoxin can be chemically coupled to functional groups in the oligopeptide, either directly or by means of a di-functional coupling agent. The choice of coupling agent or method will be well within the abilities of the ordinary person skilled in the art, see for example WO 88/00583 incorporated herein by reference, so also will be other suitable cytotoxic reagents besides the ricin and ricin derivatives already mentioned.

The detailed preparation of radioactively labelled peptides according to this invention is illustrated by the following example:

EXAMPLE

Preparation of radioactively labelled ^{125}I-YLDVY, ^{125}I-LDVGGGGSY, and ^{125}I-YGGLDVGLDVGGY

The oligopeptides YLDVY, LDVGGGGSY (corresponding amino acid sequences, see above), and YGGLDVGLDVGGY (amino acid sequence, see SEQ ID No. 2), were synthesised by standard procedures using an automatic peptide synthesizer.

Iodogen tubes were prepared by dissolving Iodogen (1,3,4,6-Tetrachloro-3α,6α-diphenylglycouril) in chloroform at a concentration of 1 mg.ml^{-1}. Aliquots of 50µl (50µg Iodogen) were dispensed into polypropylene cryo-tubes and the chloroform evaporated to dryness. These tubes were then stored desiccated at -20°C until required.

Prior to radio-labelling the peptides were dissolved in phosphate buffered saline (PBS) at a concentration of 50µg.ml^{-1}.

Iodogen tubes were equilibrated to room temperature before the addition of 200µl peptide solution and 1-10µl of ^{125}I (in aqueous solution). The reaction mixture was then left for 15 minutes at room temperature with occasional shaking. Following the incubation period the reaction mixture was removed and passed through a Sephadex G10 column which had been equilibrated with PBS. The column, which separates radio-labelled peptide from free iodine was eluted with PBS and 2ml fractions collected. radioactivity in the fractions was measured and the eluted peptides, represented by the first radioactive peak from the column, collected and stored at 4°C until required.

At this point, it may be mentioned that, in accordance with the usual convention on the representation of labelled peptides, the representation ^{125}I-YLDVY, for example, merely indicates that the peptide YLDVY has been labelled with ^{125}I. It is not intended to indicate either the position of attachment of the label or the actual number of radioactive iodine atoms attached to the peptide molecule.

The capacity of the radioactively labelled peptides to bind to tumour cells in vitro and in vivo is illustrated by the following experiments, the results of which are illustrated in the accompanying drawings.

EXPERIMENT 1

Tumour cells from tumour cell lines T47D, LoVo, HT29, HT1080,

HEp-2, EJ28 and A431 were grown to confluence on separate microtitre plates, fixed with glutaraldehyde and then incubated for 1 hour at room temperature with varying concentrations of the three ^{125}I-labelled peptides ^{125}I-YLDVY, ^{125}I-LDVGGGGSY and ^{125}I-YGGLDVGLDVGGY. The plates were then washed to remove unbound reagent and the cell residues containing the bound label were removed from each well and the residual radioactivity of the bound label measured. From the known level of radioactivity possessed by the radio-labelled peptide, the molar amount of bound peptide was determined. The results are presented in Figures 1 to 3.

EXPERIMENT 2

In vivo experiments were performed in nude mice bearing HEp-2 and HT29 tumours obtained by injecting the nude mice subcutaneously in both flanks with from 2 to 5 x 10^6 HEp-2 and HT29 tumour cells and the tumours allowed to grow for three weeks.

In these experiments the nude tumour bearing mice divided into five groups were each injected subcutaneously with the ^{125}I-labelled peptide

^{125}I-YGGLDVGLDVGGY

(SEQ ID No. 2), and the uptake of labelled peptide measured against time. For this purpose the five groups were killed at intervals of 5 minutes, 30 minutes, 1 hour, 3 hours, and 6 hours after injection. At this time the blood and tumours were separately removed, weighed and counted for radioactivity, as well as samples taken from all major organs. Figure 4 shows the retention level at intervals of 30 minutes, 1 hour, 3 hours and 6 hours expressed as a fraction of the percentage injected dose (id) per gram relative to the five minute time point. At this point peptide uptake was 10.3% id per gram in blood, 4.5% id per gram in HEp-2 tumour and 3.0% id per gram in HT29 tumour.

The data presented clearly indicates the capacity of the LDV-containing oligopeptides of this invention to bind to tumour associated LDV binding sites in vivo and therefore potentially to provide useful transport agents for the targeting of imaging and therapeutic reagents onto tumours in vivo. Not only that, but transportation and clearance

rates are rapid enabling shortened diagnosis times in the case of tumour imaging and shortened treatment times in the case of tumour therapy.

For diagnostic purposes, the radioactively labelled oligopeptides of this invention are designed for intravenous administration in a suitable liquid carrier at a single dosage rate of no more than about 100 micrograms as a practical upper limit, more usually in the range 10 to 50 µg. For therapeutic purposes, the cytotoxic oligopeptides of this invention, i.e. the LDV-containing carrier peptide conjugated or chemically linked to a cytotoxic agent, such as the ricin A-chain, will usually be employed at dosage rates in the range 1mg to 1g depending on tumour size and/or bodyweight, and will usually be administrated serially over a period of time ranging from a few hours to a few days, or in some cases over a period of several weeks. Placing these figures in perspective, and given an average human plasma volume of 2.5L, a single diagnostic injection of 100µg represents a maximum peptide plasma concentration of from about 0.078µM to about 0.15µM depending on the molecular weight of the peptide, even assuming no clearance of the peptide from the body. Correspondingly a therapeutic dosage of 1mg represents a maximum peptide plasma concentration of from about 0.78µM to about 1.5µM, again assuming no clearance of peptide from the body. At these extremely low peptide plasma concentrations, which in practice will never be attained due to the systemic clearance rates of the peptide from the body, it is highly surprising that any significant amount of LDV peptide remains bound to the tumour, let alone a sufficient amount to permit effective imaging or treatment of the tumour. Those peptide plasma concentrations may indeed be contrasted quite remarkably with the >500µM concentration recorded by Kloczewiak et al, loc. cit., as necessary to obtain 50% inhibition of fibronectin binding to activated platelets using the peptide HHLQLLKQLLDV and which figure would indicate a very low level of binding of the LDV-containing peptide to the activated platelets. In contrast to that, the LDV-containing peptides used in the present invention show a quite exceptional and quite unexpected high level of affinity for tumour cells in vivo.

Whether for diagnostic or chemotherapeutic purposes, the labelled or cytotoxic oligopeptides of this invention will be formulated in a

suitable liquid carrier for intravenous administration. Suitable carriers for this purpose include physiological saline, sterilized water, various other buffer and/or sugar or salt solutions as known in the art. For convenience of administration the concentration of labelled or cytotoxic peptide in the injectable carrier liquid will usually be in the range 1 to 30% by weight, more usually 1 to 10% by weight.

APPENDIX

Peptide sequence details.

SEQ ID No. 1

Sequence Type : Amino acid
Sequence Length : 25
Strandedness : Single
Topology : Linear
Molecular Type : Peptide
Original Source : Fibronectin CS1-sequence
Original Organism : Human
Immediate Experimental Source : Synthetic
Sequence Description:

D E L P Q L V T L P
asp-glu-leu-pro-gln-leu-val-thr-leu-pro-
H P L L H G P E I L
his-pro-leu-leu-his-gly-pro-glu-ile-leu-
D V P S T
asp-val-pro-ser-thr

SEQ ID No. 2

Sequence Type : Amino acid
Sequence Length : 13
Strandedness : Single
Topology : Linear
Molecular Type : Peptide
Original Source : -
Original Organism : -
Immediate Experimental Source : Synthetic
Sequence Description:

Y G G L D V G L D V
tyr-gly-gly-leu-asp-val-gly-leu-asp-val-
G G Y
gly-gly-tyr

CLAIMS

1. An oligopeptide consisting of from 4 to 50 peptide units containing as a triplet therein the amino acid sequence leu-asp-val (LDV) and as a result of which the oligopeptide is capable of binding in vivo with pathological tissues containing an LDV binding site, that oligopeptide having attached thereto or conjugated therewith a radioactive label or cytotoxin.

2. An oligopeptide according to claim 1, which contains from 4 to 30 peptide units.

3. An oligopeptide according to claim 1, comprising as the peptide chain the sequence SEQ ID No. 1, or a sub-sequence thereof having a length of at least four units and which contains the LDV triplet, and to which peptide chain or unit there is attached said radioactive label or cytotoxin.

4. An oligopeptide according to claim 1, comprising as the peptide chain the pentapeptide

tyr-leu-asp-val-tyr

to which is attached said radioactive label or cytotoxin.

5. An oligopeptide according to claim 1, comprising as the peptide chain the nonapeptide

leu-asp-val-gly-gly-gly-gly-ser-tyr

to which is attached said radioactive label or cytotoxin.

6. An oligopeptide according to claim 1, comprising as the peptide chain the tridecapeptide having the sequence SEQ ID No. 2, and to which is attached the said radioactive label or cytotoxin.

7. An oligopeptide according to claim 1, which is a radio-labelled

oligopeptide of 4 to 50 peptide units containing said LDV triplet.

8. An oligopeptide according to claim 7, which is a radioactively labelled oligopeptide comprising the 25 unit sequence SEQ ID No. 1 or a sub-sequence thereof containing at least four units and containing the said LDV triplet, that sequence or sub-sequence being labelled with a radioactive label.

9. An oligopeptide according to claim 1, which is a radioactively labelled oligopeptide comprising the pentapeptide

tyr-leu-asp-val-tyr

labelled with a radioactive label.

10. An oligopeptide according to claim 1, which is a radioactively labelled oligopeptide comprising the nonapeptide

leu-asp-val-gly-gly-gly-gly-ser-tyr

labelled with a radioactive label.

11. An oligopeptide according to claim 1, which is a radioactively labelled oligopeptide comprising the tridecapeptide SEQ ID No. 2 labelled with a radioactive label.

12. The radioactively labelled peptides:

^{125}I-{tyr-leu-asp-val-tyr}

^{125}I-{leu-asp-val-gly-gly-gly-gly-ser-tyr}

and ^{125}I-{SEQ ID No. 2}.

13. A diagnostic compound for <u>in vivo</u> tumour imaging comprising a radioactively labelled oligopeptide according to claim 1, wherein the label is selected from ^{99m}Tc, ^{125}I and ^{111}In.

14. An anti-tumour therapeutic compound being a compound according to claim 1 in which the said oligopeptide is chemically linked to or conjugated with a cytotoxin.

15. An anti-tumour therapeutic compound according to claim 14, wherein the cytotoxin is ricin or a ricin derivative.

16. An anti-tumour therapeutic compound according to claim 15, wherein the cytotoxin is the ricin A-chain.

17. An anti-tumour therapeutic compound according to claim 14, wherein the oligopeptide linked to the cytotoxin is selected from

i) SEQ ID No. 1;

ii) sub-sequences of SEQ ID No. 1 containing at least 4 peptide units and containing the LDV sequence;

iii) the pentapeptide: tyr-leu-asp-val-tyr;

iv) the nonapeptide: leu-asp-val-gly-gly-gly-gly-ser-tyr; and

v) the tridecapeptide: SEQ ID No. 2.

18. An anti-tumour therapeutic compound according to claim 17, wherein the said cytotoxin is ricin or a cytotoxic ricin derivative.

19. A diagnostic reagent for <u>in vivo</u> tumour imaging comprising an intravenously administrable liquid carrier containing an effective amount of a radioactively labelled oligopeptide according to claim 1.

20. A diagnostic reagent according to claim 19, wherein the radioactively labelled oligopeptide is an oligopeptide as claimed in claim 8.

21. A diagnostic reagent according to claim 19, wherein the radioactively labelled oligopeptide is an oligopeptide as claimed in claim 9.

22. A diagnostic reagent according to claim 19, wherein the radioactively labelled oligopeptide is an oligopeptide as claimed in claim 10.

23. A diagnostic reagent according to claim 19, wherein the radioactively labelled oligopeptide is an oligopeptide as claimed in claim 11.

24. A diagnostic reagent according to claim 19, wherein the radioactively labelled oligopeptide is an oligopeptide as claimed in claim 12.

25. An anti-tumour therapeutic reagent comprising an intravenously administrable liquid carrier comprising an effective amount of a cytotoxic oligopeptide according to claim 1.

26. An anti-tumour therapeutic reagent according to claim 25, wherein the cytotoxic oligopeptide is as defined in claim 15.

27. An anti-tumour therapeutic reagent according to claim 25, wherein the cytotoxic oligopeptide is as defined in claim 16.

28. An anti-tumour therapeutic reagent according to claim 25, wherein the cytotoxic oligopeptide is as defined in claim 17.

29. An anti-tumour therapeutic reagent according to claim 25, wherein the cytotoxic oligopeptide is as defined in claim 18.

30. A method of in vivo tumour imaging which comprises intravenously administering to the patient an effective amount of a radioactively labelled oligopeptide as claimed in claim 1, and radiographically detecting the bound label.

31. A method of treating tumours in vivo which comprises intravenously administering to the patient an effective amount of a cytotoxic oligopeptide as claimed in claim 1.

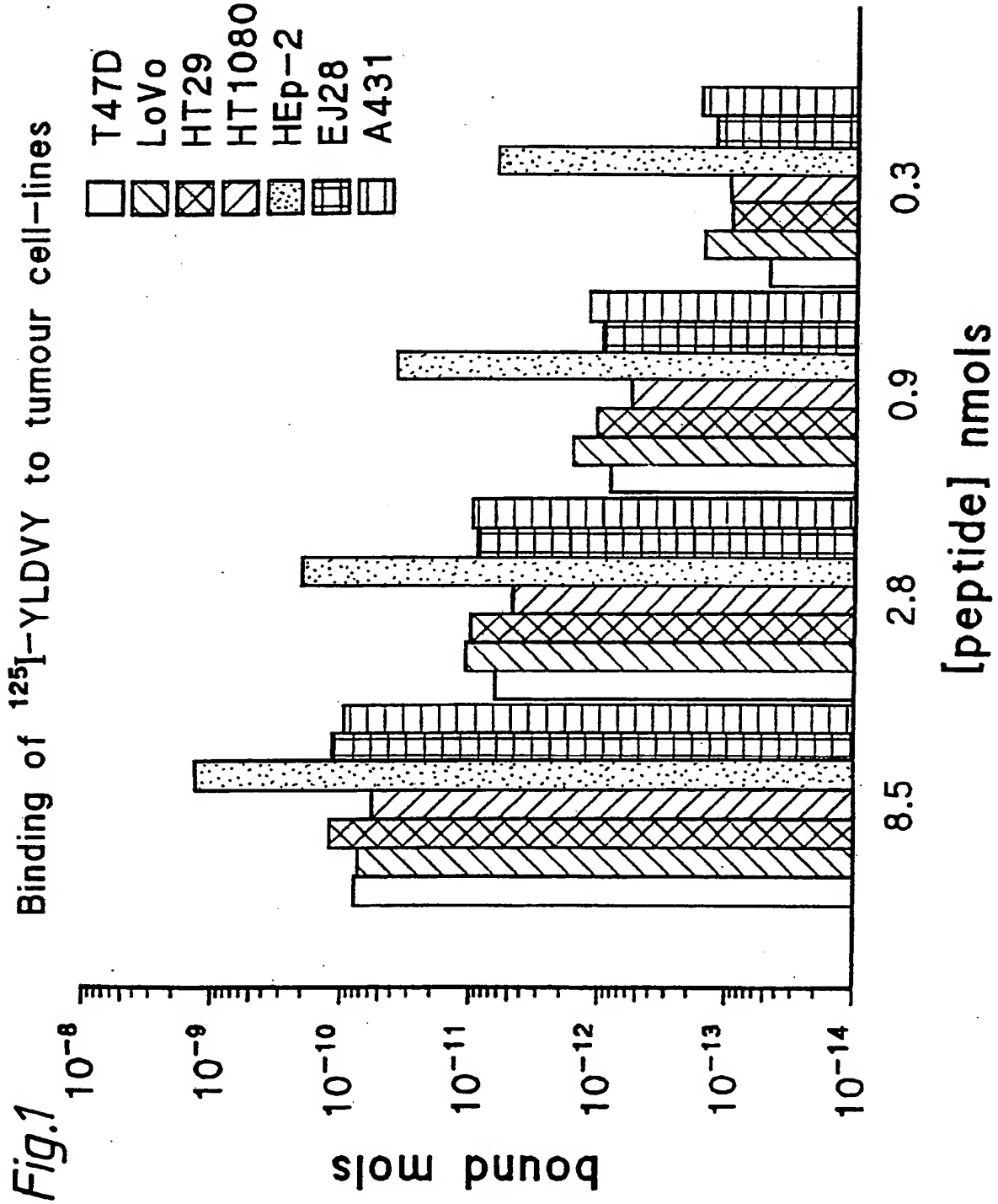
Binding of ^{125}I-YLDVY to tumour cell-lines
T47D
LoVo
HT29
HT1080
HEp-2
EJ28
A431
bound mols
10^{-8}
10^{-9}
10^{-10}
10^{-11}
10^{-12}
10^{-13}
10^{-14}
8.5
2.8
0.9
0.3
[peptide] nmols

Fig.1

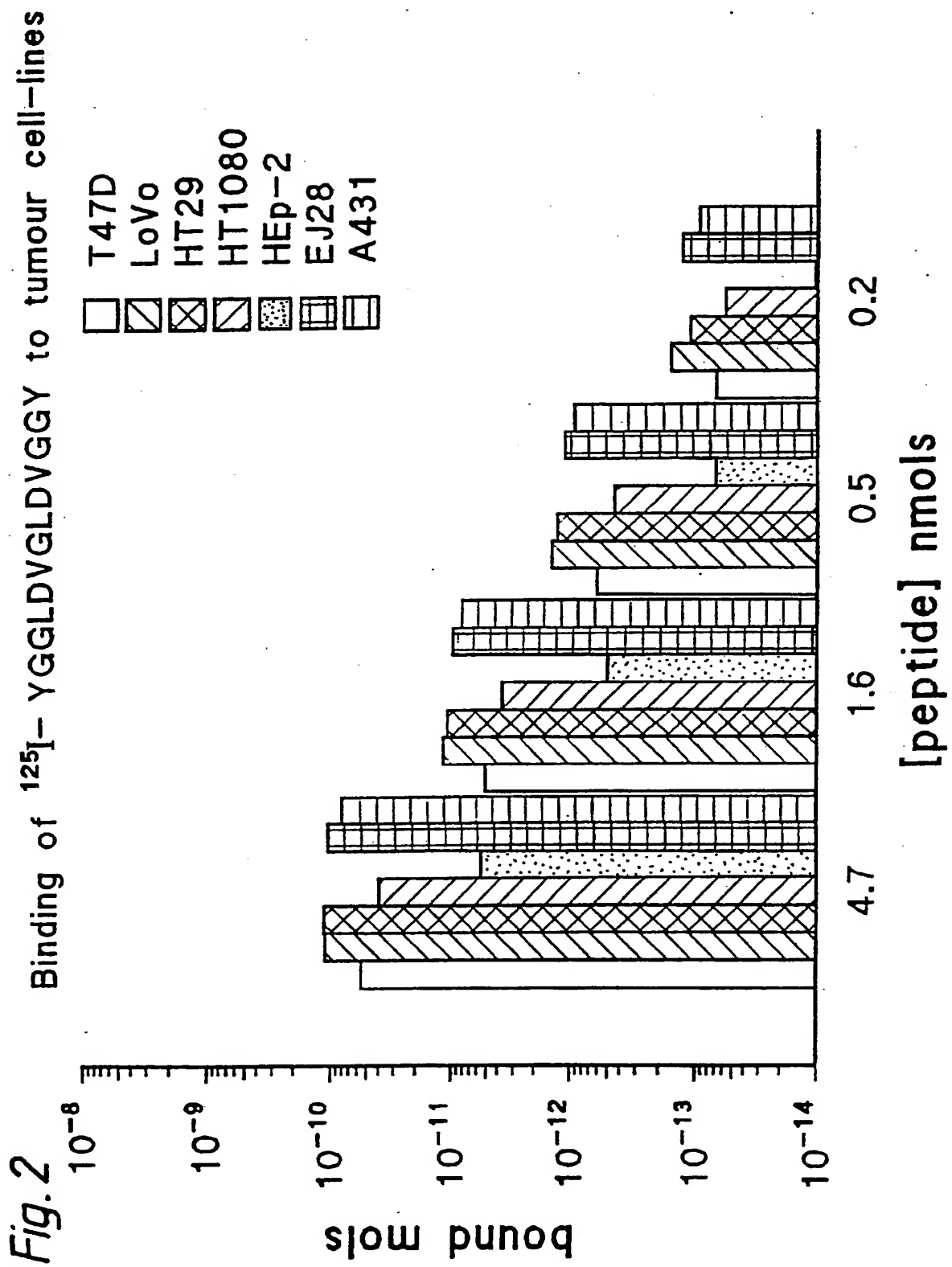
Binding of ^{125}I- YGGLDVGLDVGGY to tumour cell-lines
T47D
LoVo
HT29
HT1080
HEp-2
EJ28
A431
bound mols
10^{-8}
10^{-9}
10^{-10}
10^{-11}
10^{-12}
10^{-13}
10^{-14}
4.7
1.6
0.5
0.2
[peptide] nmols

Fig. 2

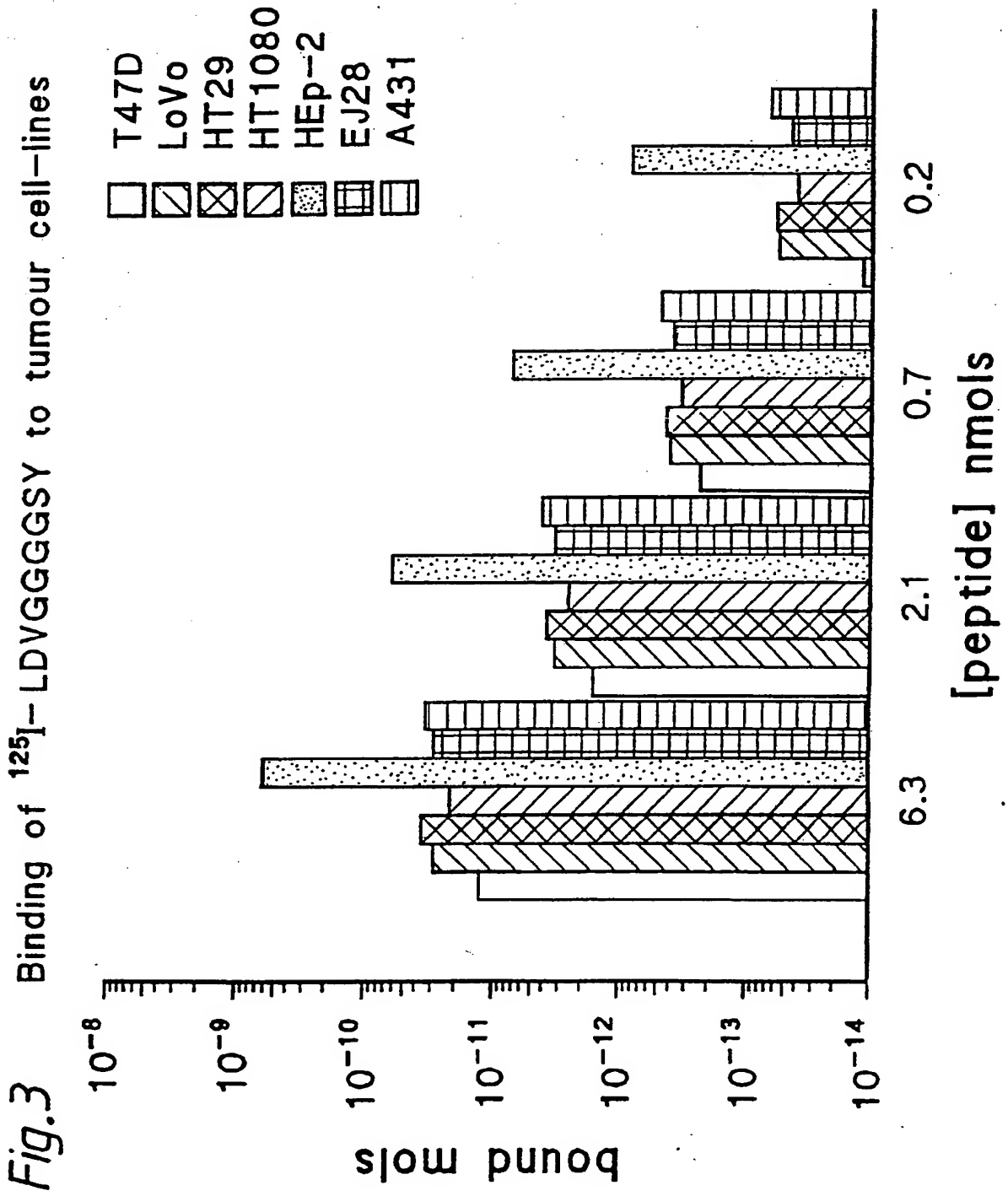
T47D
LoVo
HT29
HT1080
HEp-2
EJ28
A431
Binding of ^{125}I-LDVGGGGSY to tumour cell-lines
bound mols
10^{-8}
10^{-9}
10^{-10}
10^{-11}
10^{-12}
10^{-13}
10^{-14}
6.3
2.1
0.7
0.2
[peptide] nmols

Fig.3

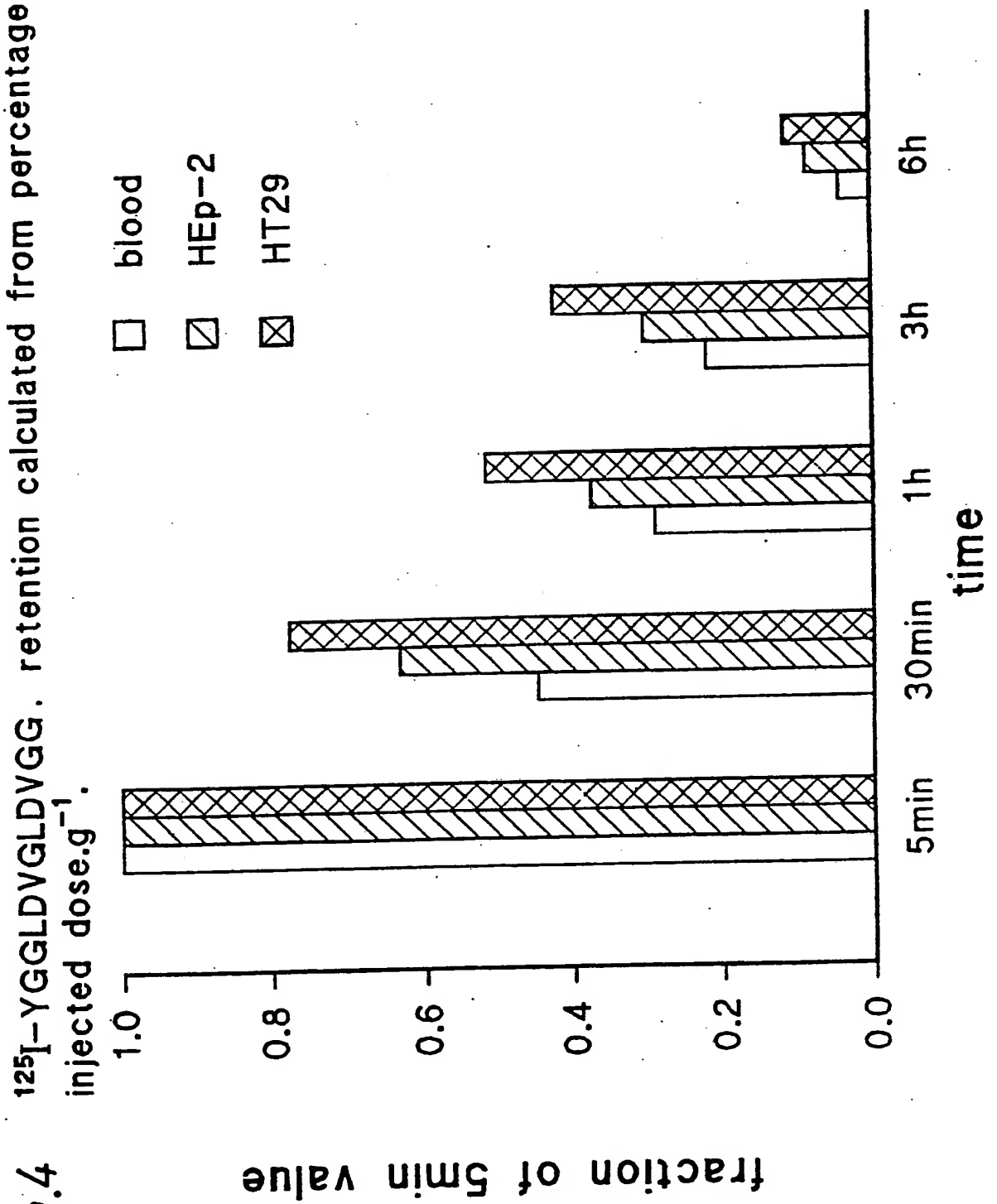
^{125}I-YGGLDVGLDVGG. retention calculated from percentage injected dose.g^{-1}.
blood
HEp-2
HT29
fraction of 5min value
1.0
0.8
0.6
0.4
0.2
0.0
5min
30min
1h
3h
6h
time

Fig.4

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 92/01458

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K47/48; A61K49/02

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
X	CHEMICAL ABSTRACTS, vol. 115 Columbus, Ohio, US; abstract no. 155972k,	1-31
Y	& BIOCHEM. SOC. TRANS vol. 19, no. 4, 1991, MANCHESTER, UK page 380S MAKAREM R. ET AL. 'LDV: A novel cell adhesion motif recognized by the integrin alpha 4 beta 1.'	1-31

-/--

° Special categories of cited documents :[10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
24 NOVEMBER 1992	07. 12. 92
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	BERTE M.

Form PCT/ISA/210 (second sheet) (January 1985)

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
Y	CHEMICAL ABSTRACTS, vol. 114 Columbus, Ohio, US; abstract no. 201960j, cited in the application & J. BIOL. CHEM. vol. 266, no. 6, 1991, MANCHESTER pages 3759 - 3785 MOULD A. ET AL. 'The CS5 peptide is a second site in the IIICS region of fibronectin recognized by the integrin alpha 4 beta 1.'	1-31
Y	CHEMICAL ABSTRACTS, vol. 115 Columbus, Ohio, US; abstract no. 177722f, cited in the application & J. BIOL. CHEM. vol. 266, no. 3, 1991, BETHESDA pages 15075 - 15079 KOMORIYA A. ET AL. 'The minimal essential sequence for a major cell type-specific adhesion site (CS1) within the alternatively spliced type III connecting segment domain of fibronectin is leucine-aspartic-valine.'	1-31
P,X	CHEMICAL ABSTRACTS, vol. 117 Columbus, Ohio, US; abstract no. 68307v, & J. CELL BIOL. vol. 116, no. 2, 1991, MINNEAPOLIS pages 489 - 497 WAYNER E.A. ET AL 'Activation-dependent recognition by hematopoeitic cells of the LDV sequence in the V region of fibronectin.'	1-31
Y	WO,A,9 015 818 (ANTISOMA) 27 December 1990 cited in the application see the whole document	1-31
A	EP,A,0 359 347 (NEORX) 21 March 1990 see page 3, line 53 - page 4, paragraph 8; claims; example IV	1-31
A	EP,A,0 428 266 (TAKARA SHUZO) 22 May 1991 cited in the application see the whole document	1-31

-/--

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
A	WO,A,9 009 799 (COLORADO STATE UNIVERSITY) 7 September 1990 see the whole document -----	1-31

INTERNATIONAL SEARCH REPORT

International application No.
PCT/GB92/01458

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
ALTHOUGH CLAIMS 30; 31 ARE DIRECTED TO A DIAGNOSTIC METHOD PRACTISED ON THE HUMAN/ANIMAL BODY THE SEARCH HAS BEEN CARRIED OUT AND BASED ON THE ALLEGED EFFECTS OF THE COMPOUND/COMPOSITION.

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 9201458 SA 63142

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 24/11/92 The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A-9015818	27-12-90	EP-A-	0429626	05-06-91
		GB-A-	2241243	28-08-91
		JP-T-	4505330	17-09-92
EP-A-0359347	21-03-90	US-A-	5135736	04-08-92
		JP-A-	2124833	14-05-90
EP-A-0428266	22-05-91	JP-A-	4059734	26-02-92
		JP-A-	4077435	11-03-92
		JP-A-	3127742	30-05-91
WO-A-9009799	07-09-90	AU-A-	5186090	26-09-90